in controls (8.0% vs 2.9%, $P = 0.02$). Individuals with the referred genotype were under a 4.30-fold (95% CI: 1.22–15.14) increased risk for MM than others. In addition, we observed an excess of the *P53* Arg/Arg wild-type genotype in patients who were highly exposed to sunlight compared to those who were protected against UV radiation (70.0% vs 45.2%, $P= 0.02$) and also compared to controls (70.0% vs 47.4%, $P = 0.006$). Individuals with this genotype and highly exposed to sunlight had a 2.58-fold (95% CI: 1.61–38.42) increased risk for MM. Moreover, the frequency of the combined *XPF* genotype TC+CC and *P53* Arg/Arg+Arg/Pro was higher in patients with light skin than in patients non-light skin (94.0% vs 50.0%, $P = 0.03$).

Conclusions: The data suggests that the *XPC* A2920C, the *XPF* T30028C and the *P53* Arg72Pro polymorphisms, alone or in combination, alter the risk for MM and its clinical manifestation in our region. We believe that carriers of specific genotypes of the referred genes should receive additional recommendation to avoid exposition to sunlight and should be frequently evaluated by a dermatologist with the purpose of to perform an early diagnosis of the disease.

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9309 POSTER

Signalling of the Major Histocompatibility Complex (MHC) Class II Molecules in Melanoma Cells

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Background: Melanoma is the cancer with the higher incidence in western populations and is notoriously resistant to all current cancer therapy. Indeed, the reason of the limited success of immunotherapeutic approaches could be the ability of melanoma cells to escape immune response and alter the function and survival of immune cells. Interestingly, almost 50% of metastatic melanoma, in contrast to skin melanocytes, expresses constitutively the major histocompatibility complex (MHC) class II which is associated to disease progression and is linked to a poor prognosis. The MHC class II molecules during T cell/ professional antigen-presenting cells (APCs) interactions are localised, as signalling receptors, to membrane lipid rafts which are thought to be sites where transmembrane signalling complexes assemble. In the aim to understand the molecular mechanisms used by melanomas to frustrate an effective anti-tumour response, we studied in MHC class II constitutive expressing melanoma cell lines, the membrane localization of the class II molecules as well as the MHC class II signalling.

Material and Methods: The class II constitutive expressing melanoma cells (A375 and HT144 cell lines) were stimulated with a specific anti-HLA-DR mAb (L243) that mimics the TCR interaction with the class II molecules or left unstimulated. The lipid rafts of stimulated and unstimulated melanoma cells were isolated by discontinuous sucrose gradient and analysed by western blot. Exosomes secreted by stimulated and unstimulated melanoma cells were purified and analysed by western blot.

Results: Within the hypothesis that MHC class II constitutive expressing melanoma cells might mimic an APC, we stimulated the HT144 melanoma cells with L243 monoclonal antibody and we isolated the lipid rafts. In these membrane domains of stimulated HT144 cells we observed an increased localisation of HLA-DR molecules as reported for A375 cells. Therefore, we compared the expression level of some adhesion receptors as well as the exosomes secreted by stimulated and unstimulated A375 and HT144 melanoma cells.

Conclusions: Therefore, our results underline the role played in melanoma cells by the MHC class II dependent signalling on motility and exosomes functionality. The study of the signalling activated by class II molecules could help to elucidate how affect the metastatic dissemination ability of melanoma cells as well as the role of exosomes on the microenvironment of tumour sites.

9310 POSTER

Benzo[c]phenanthridine Alkaloids – Compounds With High Anti-proliferative Activity Against Malignant Melanoma

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Background: Malignant melanoma (MM) ranks amongst the most aggressive and therapy resistant human cancers and its incidence has been steadily increasing. Therefore, identification of new drugs with therapeutic potential towards melanoma could be of great significance. We have studied the biological activities of benzo[c]phenanthridine alkaloids (BAs), a group of natural products with significant anti-proliferative activities.

Material and Methods: The mechanisms of anti-proliferative effects of BAs were investigated using MTT assay, flow cytometry, Western blot analysis, fluorescence and electron microscopy.

Results: We have analyzed the cellular responses to sanguinarine (SA), chelerythrine (CHE), chelidonine (CHLD), sanguilutine (SL) and chelilutine (CHL) on a model melanoma cell line A375 and two A375-derived cell lines with defect in the p53 pathway. In our assays, all tested alkaloids exerted strong anti-proliferative effect on all three cell lines, suggesting that their anti-proliferative activity does not require functional p53 despite of the fact that all alkaloids caused DNA damage, which was demonstrated by induction of H2AX phosphorylation. While CHE and CHL was assessed as the most potent apoptosis inductors, SA, in spite of having a greater anti-proliferative activity, induced apoptosis to a much lesser extent. CHLD was the least effective inductor of apoptosis. The activation of apoptosis by these BAs was accompanied by a cleavage of caspase-3 and PARP, changes in mitochondrial membrane potential (MMP) and a decrease in the cellular levels of anti-apoptotic proteins Bcl-xL, Mcl-1 and XIAP. In contrast, SL treatment of melanoma cells resulted only in a change of MMP and decrease in the levels of the above listed anti-apoptotic proteins. Furthermore we have observed vacuolization of cytoplasm indicating autophagy and as type of cell death induced by SL, we have assessed necroptosis, a caspase independent cell death.

Conclusions: Our results suggest that individual BAs are able to activate various types of cell death in malignant melanoma cells, regardless of their p53 status, indicating that these compounds might also have therapeutic potential for the treatment of the various types of cancer in which the function of the p53 pathway is commonly impaired and the ability to induce caspase independent cell death could be advantageous especially for the treatment of apoptosis resistant tumour cells.

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9311 POSTER

Topical Application of Lantadene A and its Methyl Ester (LAM) Inhibit Carcinogenesis and Induce Apoptosis in UVB Induced Skin Tumours in Mice

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Background: Lantadene A (LA) is a pentacyclic triterpenoid isolated from weed *Lantana camara* L. and its semi-synthetic analogue LAM has shown squamous cell carcinogenesis inhibition in various models. The purpose of this study is to evaluate topical effect of LA and LAM on tumorigenesis in UVB-pretreated high-risk mice.

Material and Methods: SKH-1 hairless mice were irradiated with ultraviolet B (UVB) twice weekly for 20 weeks. These tumour-free mice, which had a high risk of developing skin tumours during the next several months, were then treated topically with Lantadene A (LA; 85 nmol) and Lantadene A methyl ester (LAM; 85 nmol) once a day 5 days a week for 18 weeks in the absence of further treatment with UVB.

Results: Topical applications of LA to these mice decreased the number of nonmalignant and malignant skin tumours per mouse by 38% and 42%, respectively. Topical applications of LAM decreased the number of nonmalignant and malignant tumours per mouse by 35% and 46%, respectively. Immunohistochemical analysis showed that topical applications of LA and LAM increased apoptosis as measured by the number of caspase 3-positive cells in nonmalignant skin tumours by 76% and 62%, respectively, and in squamous cell carcinomas by 72% and 56%, respectively, but there was no effect on apoptosis in non tumour areas of the epidermis. Topical applications of LA and LAM had a small inhibitory effect on proliferation in non malignant tumours as measured by BrdUrd labeling (26−32%), and there was also a similar, but non significant, inhibitory effect on proliferation in malignant tumours.

Conclusion: The results suggest a need for further studies to determine whether topical applications of LA and LAM can inhibit sunlight-induced skin cancer in humans.